

# RLF (D-9): sc-166128

## BACKGROUND

c-Jun is an important transcription factor that is involved in the regulation of proliferation, differentiation and cellular transformation induced by oncogenic Ras. An activated Ras effector, RLF (also designated as Ras-associated protein Rab2L/RalGDS-like factor), a guanine nucleotide exchange factor (GEF) of the small GTPase Ral, induces the phosphorylation of serines 63 and 73 of c-Jun. The RalGEF-Ral pathway plays an important role in Ras-dependent c-Jun phosphorylation. RLF functions as an intermediate between Ras and Ral pathways by binding to the GTP-bound form of Ras proteins through its C-terminal Ras-binding domain (RBD), which is very similar to that of RalGDS- RBD. RLF-induced Ral activation is stimulated by Ras. RLF, when targeted to the plasma membrane using the Ras farnesyl attachment site (RLF-CAAX), is constitutively active to induce both Ral activation and c-Fos promoter activity. RLF mediates a distinct Ras-induced signaling pathway to gene induction and RLF-CAAX stimulates both transcriptional activation and cell growth. Overexpression of RLF-CAAX induces neuroretina cell division, but has no effect on ERK activity, whereas inhibition of MEK blocks both Ras- and RLF-CAAX-induced differentiation, suggesting that RalGEFs induce differentiation depending on the basal MEK or ERK activity.

## REFERENCES

1. Wolthuis, R.M., et al. 1997. Stimulation of gene induction and cell growth by the Ras effector RLF. *EMBO J.* 16: 6738-6761.
2. Esser, D., et al. 1998. Structure determination of the Ras-binding domain of the Ral-specific guanine nucleotide exchange factor RLF. *Biochemistry* 37: 13453-13462.
3. Wolthuis, R.M., et al. 1998. Ras-dependent activation of the small GTPase Ral. *Curr. Biol.* 8: 471-474.
4. Verheijen, M.H., et al. 1999. Interdependent action of RalGEF and Erk in Ras-induced primitive endoderm differentiation of F9 embryonal carcinoma cells. *Oncogene* 18: 4435-4439.
5. de Ruiter, N.D., et al. 2000. Ras-dependent regulation of c-Jun phosphorylation is mediated by the Ral guanine nucleotide exchange factor-Ral pathway. *Mol. Cell. Biol.* 20: 8480-8488.
6. Sood, R., et al. 2000. The human RGL (RalGDS-like) gene: cloning, expression analysis and genomic organization. *Biochim. Biophys. Acta* 1491: 285-288.

## CHROMOSOMAL LOCATION

Genetic locus: RGL2 (human) mapping to 6p21.32.

## SOURCE

RLF (D-9) is a mouse monoclonal antibody raised against amino acids 131-250 mapping within an internal region of RLF of human origin.

## PRODUCT

Each vial contains 200 µg IgG<sub>1</sub> kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

## APPLICATIONS

RLF (D-9) is recommended for detection of RLF of human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Suitable for use as control antibody for RLF siRNA (h): sc-41846, RLF shRNA Plasmid (h): sc-41846-SH and RLF shRNA (h) Lentiviral Particles: sc-41846-V.

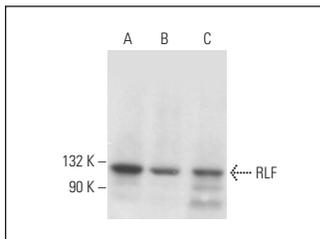
Molecular Weight of RLF: 84 kDa.

Positive Controls: Caki-1 cell lysate: sc-2224, HEK293 whole cell lysate: sc-45136 or RLF (h): 293T Lysate: sc-115319.

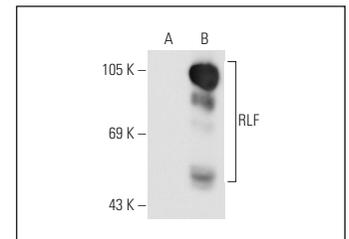
## RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## DATA



RLF (D-9): sc-166128. Western blot analysis of RLF expression in Caki-1 (A), HEK293 (B) and MIA PaCa-2 (C) whole cell lysates.



RLF (D-9): sc-166128. Western blot analysis of RLF expression in non-transfected: sc-117752 (A) and human RLF transfected: sc-115319 (B) 293T whole cell lysates.

## STORAGE

Store at 4° C, \*\*DO NOT FREEZE\*\*. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.